Stay Positive, We've Got It Under Control: The Testing of a Positive Control for a Beta-Lactamase Experiment

GRP 1
Section 1

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ABSTRACT

The purpose of this experiment was to identify predicted beta-lactamase genes, genes which code for enzymes that digest beta-lactam class antibiotics, in *Elizabethkingia miricola*. Our group was given the positive control, a gene from *Elizabethkingia meningoseptica* which has already been identified as a beta-lactamase gene. We designed forward and reverse primers for this gene, which were used in PCR, polymerase chain reaction, in order to replicate this gene. After determining that PCR had been successful using gel electrophoresis, we cloned the gene in *E. coli* and then tested these bacteria for beta-lactam resistance. We determined that our sample of *E. coli* did contain genes which express beta-lactam resistance, which is the result we expected as the positive control.

INTRODUCTION

The *Elizabethkingia* genus is a gram-negative bacteria consisting of four species: *Elizabethkingia meningoseptica, Elizabethkingia anophelis, Elizabethkingia miricola,* and *Elizabethkingia endophytica.* These species contain numerous beta-lactamase genes, the genes which code for beta-lactamase enzymes. These enzymes digest beta-lactam antibiotics, a broad category of antibiotics which contain a beta-lactam ring in their molecular structures. This can make *Elizabethkingia* bacteria very difficult to eradicate as they resist many commonly used antibiotics. A number of beta-lactamase genes have been identified within *Elizabethkingia* bacteria, but numerous more remain to be identified.

RESULTS

The results of the testing of our sample of *E. coli* filled with recombinant DNA showed that our sample did in fact possess beta-lactam resistance. This is the result that we expected, as our sample was the positive control for the experiment. If our results had turned up negative, it would have meant that some error had been made, or that the experiment had been designed improperly, and so the results would not have been trustworthy. However, the results that we received are proper, and so the results of the experiments on the predicted beta-lactamase genes of *Elizabethkingia miricola* can be trusted.

Image 3 below shows the final results of our experiment. Our sample is the one next to the label for group one. It appears dark red in color, contrasting against the negative sample beside it. This means that our gene expressed beta-lactam resistance very strongly.

Image 3

DISCUSSION

We found that our sample of DNA did express beta-lactam resistance. This was the correct result as we had the positive control. Because we found that our sample was positive, and because both the negative control and the empty vector were found to be negative, the results of the experiment are trustworthy. This means that whatever genes of *Elizabethkingia miricola* were found to be positive are beta-lactamase genes and that the ones that were negative were not beta-lactamase genes.

The results of the tests on the *Elizabethkingia miricola* genes serves to further our knowledge of beta-lactamases and the mechanism by which they have arisen. From this, we can learn better ways to treat those who are infected with these bacteria.

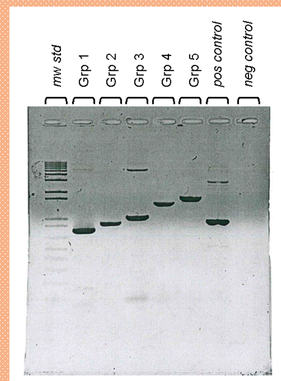
While the experiment that we performed contributed no new knowledge, it was vital to the overall experiment, as using controls in experiments is fundamental to good science. So while we have no data upon which to make conjecture, the experiment which we performed gives validity to those of the other groups.

Materials

- E. coli Cells
- Ligation mixture
- Ice
- Warm water (42° C)
- Recovery broth
- P20 Micropipetter
- Disposable tips
- dH2O
- Ligase Buffer
- Linear Plasmid Vector
- PCR Product
- T4 DNA Ligase Enzyme
- Petri dishes

Methods

- PCR – polymerase chain reaction uses the primers with taq polymerase enzyme to synthesize the desired gene in bulk.
- Gel Electrophoresis – using samples of agarose gel, we inserted a small amount of our sample and put it through electrophoresis to determine if PCR was successful. The results of this process are shown in image 1.
- Cloning – we cloned our sample in *E. coli* bacteria and then tested these bacteria to see if they expressed beta-lactam resistance. The tray containing these bacteria is found in image 2.



Gel 1

Group	Sample
1	E.men_bla557
2	E.mir_strep257
3	E.mir_bla1019
4	E.mir_bla1527
5	E.mir_bla1534
PCR Pos control	E.mir_chlor174
PCR Neg control	E.mir_chlor174 no primers

Image 1

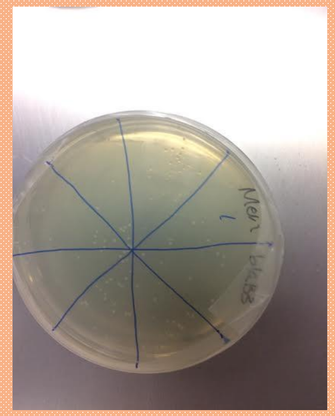


Image 2

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